UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 13, 2000

MEMORANDUM

SUBJECT: PC# 032501; Acute Delayed Neurotoxicity and NTE Studies in Hens with Disulfoton.

DP Barcode: D262076 Submission Code: S5730026
Chem Tox#: 341 Rereg Case#: 0102

From: David G Anderson, PhD
Reregistration Branch-2
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Thru: Alan Nielsen BSS
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The registant submitted a require repeat study of an Acute Delay Neurotoxicity Study in Hens with a NTE Study. The citation and Executive Summary is below and the DER is attached.

CITATION: Andrews, P and Popp, A (1999) S S276(c.n.: Disulfoton) Study for Delayed neurotoxicity following Acute Oral Administration to Hens, EPA Guideline 81-7, Bayer Report No. 109423. 75 pages. November 5, 1999. MRID 44996401. Unpublished.

EXECUTIVE SUMMARY: In an acute delayed neurotoxicity study in hens (MRID# 44996401), disulfoton was acutely administered orally to 18 LSL laying hens at 40 mg/kg bird in a single dose. Fifteen hens were used as controls. Doses were administered in aqueous 2% Cremophor at 5 ml/kg bird. Five to 18 minutes before administration of the disulfoton, atropine was administered s.c. (0.5 ml/kg of 4% atropine sulfate). Directly prior to the administration of the disulfoton, 0.5 ml/kg of 10% atropine sulfate and 10% 2-PAM chloride was injected s.c. The afternoon of day 0, 0.5 ml/kg of 5% atropine sulfate and 5% 2-PAM chloride was injected s.c. and again the morning and afternoon of day 1. Clinical observations were made at least daily.

Forced motor activity tests were conducted by forcing the hens to run around a 12-13 m^2 area and rated for coordination, ataxia, and paresis. NTE studies were conducted at 24 and 48 hours on the spinal cords, sciatic nerves and ½ of the brain in each of 3 hens per group. Cholinesterase activity studies were conducted on the other ½ of the brain from each bird in the NTE study at 24 and 48 hours post treatment. The study was conducted at 1.4 times the LD50 for hens.

No typical signs of organophosphate induced delayed neuropathy was seen during the study or on microscopic examination of the treated birds at termination at 3 weeks. No inhibition was seen in the NTE study at 24 hours or 48 hours. Inhibition was low between 4% and 8% and was not considered to be indicative of OPIDP. Cholinesterase activity in the brain was inhibited 83% and 59% at 24 and 48 hours, respectively.

No hens died, but by day 7 there was a decrease in body weight of over 5%. The hens slowly recovered and by the end of 3 weeks, body weight of the treatment group and of the controls did not differ.

Severely uncoordinated gait was observed in all treated birds within 5 minutes of being dosed with atropine and before disulfoton treatment. The report authors attributed this abnormal gait to atropine since it lasted only for the duration of the atropine treatment (2 days). However, the report authors also noted reduced motility in 1-3 birds for 0-1 day, which they attributed to disulfoton treatment. Neither statements are completely supportable because the hens were dosed with atropine and disulfoton during most of this period. However, the temporary uncoordinated gait was followed by no microscopic findings in nerve tissue and no other signs, which supports a conclusion of no demonstrated OPIDP in hens dosed with disulfoton.

Microscopic examination of the test birds showed 3 (25% - 8% in each region, grade 1) lesions in treated birds and 1 (11%, grade 1) in the same control brain regions. Since these lesions were similar to those found in controls from previous studies, they were considered incidental.

The study supports a conclusion the disulfoton does not cause acute delayed neuropathy (OPIDP) in hens.

The study is acceptable for an acute delayed neurotoxicity study (OPPTS# 870.6100) in hens.

SignOff Date:	1/13/00
DP Barcode:	D262076
HED DOC Number:	013957
Toxicology Branch:	RRB2

EPA Reviewer: David Anderson, Ph.D.
Reregistration Branch 2/HED (7509C)

EPA Secondary Reviewer: Robert Fricke, Ph.D. _____

Toxicology Branch 1/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Delayed Neurotoxicity in Hens
OPPTS Number: 870.6100 OPP Guideline Number: §81-7

DP BARCODE: D262076 SUBM. CODE: S573026
P.C. CODE: 032501 TOX. CHEM. NO.: 341

TEST MATERIAL (PURITY): S 276,. Disulfoton, technical (95.9% a.i.)

SYNONYMS: Di-Syston®

CITATION: Andrews, P and Popp, A (1999) S S276(c.n.: Disulfoton) Study for Delayed neurotoxicity following Acute Oral Administration to Hens, EPA Guideline 81-7, Bayer Report No. 109423. 75 pages. November 5, 1999. MRID 44996401. Unpublished.

SPONSOR: Bayer Corp., Agriculture Division, 8400 Hawthorn Road, Kansas City, MO 64120-0013. Telephone: 816-242-2000. Dr. Premjit Halarnkar (816-242-2331) contact.

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The study supports a conclusion the disulfoton does not cause acute delayed neuropathy (OPIDP) in hens.

The study is acceptable for an acute delayed neurotoxicity study (OPPTS# 870.6100) in hens.

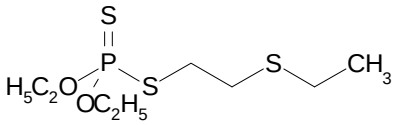
COMPLIANCE: Signed and dated Data Confidentiality, GLP, Quality Assurance, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: S276, Disulfoton
   Description: Liquid
   Lot/Batch #: 278685051
   Purity: 95.9 % a.i.
   Storage: Room temperature
   CAS #: 298-04-4

Structure:



2. Antidote 1: Atropine sulfate hydrate

3. Antidote 2: Pyridine-2-aldoxime 1-methochloride (2-PAM chloride)

4. Vehicle: 5 ml/kg aqueous 2% Cremophor® EL.

5. Test animals: Species: Laying hens

Strain: Ico: LSL breed
Age and weight (means) at study initiation: 36 weeks old; 1.46 - 1.83 kg
Source: Brinkschulte GmbH & Co KG, 48308 Senden, Germany
Housing: Floor pen with wood shavings
Diet: Ssniff Legehennen, Zucht, 4 mm, V-Alleinfutter fur Legehennen produced by Ssniff Spezialdiaten GmbH, 59494, Soest, Germany

Water: Tap water, ad libitum.
Acclimation period: 7 days
Environmental conditions:
Temperature: Approximately 23-26°C
Humidity: Approximately 40-65%
Air changes: 10-15/hour
Photoperiod: 12 hr dark/12 hr light

B. STUDY DESIGN:

1. In life dates - start: 8/2/99 end: 8/25/99

2. Animal assignment: Birds were assigned by a body weight-dependent, computer-generated randomization procedure to treatment groups as indicated in Table l. Six birds/group (3/group at each time) were scheduled for determination of NTE activity assays in nervous tissue (24 and 48 hr after administration) and AChE determination in the brain.

Table 1: Study design for OPIDP, NTE and AChE determinations

Dose administered (mg/kg) for main study	Treatment	Number of hens	Wing Numbers
0	Vehicle control	15	1-15
40	S 276	18	19-33

3. Dose selection: Dosages were reported to be selected based on the results of previous toxicity studies in which 1 of unprotected 3 hens died from a

dose of a 25 mg/kg. A previous study had shown that the acute oral LD50 of unprotected hens was 28 mg/kg. The hens tolerated a dose of 40 mg/kg when protected by atropine and 2-PAM chloride.

Based upon this consideration, the doses summarized in Table 1 were selected for the study.

4. Dosing suspensions and analysis: Homogeneity and stability of the test material was studied at 5 and 10 mg/ml and found to be homogeneous (96.9 and 94.5 % of nominal) and stable at room temperature for at least 2 hours. Dosing suspensions required stirring for the analytical procedure.

   The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

5. Statistics: No statistical analyses was presented, except body weights were analyzed by Dunnett's test.

C. METHODS:

1. Test material Administration: Subcutaneous administration of 0.5 ml/kg of 4% aqueous atropine sulfate was administered 5-18 minutes before being orally dosed with disulfoton. Directly prior to with disulfoton, 0.5 ml/kg of a solution of 10% atropine sulfate and 10% 2-PAM chloride was administered to bird. The antidote (0.5 ml/kg of a solution of 5% atropine sulfate and 5% 2-PAM chloride was repeated in the afternoon of day 0 and the morning and afternoon of day 1. All doses were stirred during treatment.

2. Observations: Birds were inspected for clinical signs and mortality at least once daily. Clinical signs was recorded as they appeared during the first 2 days of dosing. Deviations from the normal state were recorded. Particular attention was directed to the following nervous system functions, locomotions and physiological functions:

External appearance: feathers, skin color, edema, eyes, lacrimation, nasal discharge, salivation, etc.

Behavior: grooming of feathers, vocalization, agitation, aggression, cannibalism, etc.

Nervous system: apathy, motility, reflexes, gait (eg., ataxia, paralysis), cramps, tremors. etc.

Circulation: as far as evaluable: cardiac rate, pallor, etc.

Respiration: rate and depth of respiration

Posture: abnormal, lateral recumbency
Gastrointestinal function: appearance and consistency of feces, etc.

3. Clinical Neurotoxicity tests: Forced motor activity tests were conducted by forcing the hen to run an around area approximately 12-13 m^2 for about 2-3 minutes one to two times a week. During this time motor coordination, ataxia, paresis was rated.
   0 = normal
   1 = slightly abnormal gait
   2 = ataxia/disturbance of motor coordination
   3 = severe ataxia/paresis (frequent buckling of legs, collapse of the hen)
   4 = complete paralysis (inability to run).

4. Neuropathy Target Esterase (NTE) Activity: The study design for the NTE study is presented in Table 2. Six hens were randomly selected from each group listed in Table 1, and decapitated for NTE determination at 24 (3 hens) and 48 hours (3 hens) post treatment. (The 24 hour selection was stated to be 2 hours, but this was believed to be a typographical error.) Hens were preselected for determination, however, this random preselection could be over ridden by the use of the most severely affected hens. NTE was determined in the sciatic nerve, spinal cord, and right half of the brain.

5. Cholinesterase Activity: Cholinesterase activity was determined in the left half of the brains from each of the hens decapitated for the NTE determination (Table 2).

Table 2: Study design for NTE and the AChE studies

Treatment	Dose (mg/kg)	Hen numbers	
		24 hr	48 hr
Vehicle control	0	1,2,3	4,5,6
S 276	40	26,28,32	16,17,18

6. Body weight and body weight gains: Weights were recorded weekly for the first 3 weeks.

7. Food consumption: Food consumption was not determined.

8. Necropsy and Histological Examination: All surviving birds

anesthetized with nembutal at the end of the 3 weeks, exsanguinated by perfusion with phosphate buffer and perfused with 105 formalin. The following tissues were removed and fixed in 10% formalin for histological examination: sciatic nerve with branches in the tibia and lateral peroneal nerves, brain and spinal cord (cut in to 3 parts).

II. RESULTS

A. Observations

1. Acute Toxicity - No clinical signs were seen in the control group. The administration of atropine to the treated animals cause bristle plumage, uncoordinated gait and tachypnea within 5 minutes of injection. These clinical observations on the treated group disappeared on study day 2, i.e., 1 day after the last antidote administration. The report states that this effect can not be attributed to disulfoton administration, presumably because of the onset of effects immediately after atropine administration, and prior to disulfoton, but no further explanation was given. Clinical signs observed are presented in Table 3.

Table 3: Selected clinical signs in hens acutely dosed with disulfoton and followed 3 weeks.[a]

Dose mg/kg	0	40 mg/kg	
Main Study (Terminated 3 weeks after dosing)			
Number of hen/group	9	12	
Duration/# birds affected		Duration/# birds	Maximal intensity/# birds
Bristle plumage	0/0	0-2/12	2/12
Uncoordinated gait	0/0	0-2/12	2/12
Tachypnea	0/0	0-2/12	2/12
Reduced motility	0/0	1/2	2/2
Reduced motility	0/0	0/1	2/1
Dose mg/kg	0	40 mg/kg	
NTE study (Terminated 24 hours after dosing)			
Number of hens per group	3	3	
Sign	Duration in days (# birds affected)	Duration in days/ (# birds affected)	Maximal intensity/ (# birds affected)
Bristle plumage	0 (0)	0-1 (3)	2 (3)
Uncoordinated gait	0 (0)	0-1 (3)	2 (3)
Tachypnea	0 (0)	0-1 (3)	2 (3)

Reduced motility	0 (0)	0-1 (3)	2 (3)
NTE study (Terminated 48 hours after dosing)			
Number of hen per group	3	3	
Sign	Duration in days (# birds affected)	Duration in days (# birds affected)	Maximal intensity/ (# birds affected)
Bristle plumage	0 (0)	0-2 (3)	2 (3)
Uncoordinated gait	0 (0)	0-2 (3)	2 (3)
Tachypnea	0 (0)	0-2 (3)	2 (3)
Reduced motility	0 (0)	0 (0)	0 (0)

[a] Data obtained from the study report, pages 34 and 35.

B. Clinical Neurotoxicity Tests: The results of the forced motor activity showed no effects 2 days after treatment. (At least no effects were reported.) The report stated that no signs of OPIDP were seen in the any of the treated or untreated hens (Table 4). Assessment in the neurotoxicity tests (forced motor activity) were not conducted for the first 2 days after treatment because of the effects of atropine.

It should be noted that clinical observation showed uncoordinated gait up to 2 days after atropine and disulfoton treatment, the report authors attributed the bristle plumage and the uncoordinated gait to atropine treatment presumably because it started 5 minutes after atropine treatment and continued for the duration of atropine treatment for 2 days.

Table 4: Results of force motor activity on control hens and hens treated with disulfoton/atropine/2-PAM at 40 mg disulfoton/kg, expressed as number of hens with monitored parameters. [a]

# of hens	Days after treatment						
# Hens	0	2	6	9	13	16	20
Vehicle control							
3 (24 hr)	0	*	0	0	0	0	0
3 (48 hr)	0	*	0	0	0	0	0
9 (3 wk)	0	0	0	0	0	0	0
40 mg/kg treated group							
3 (24 hr)	-	*	0	0	0	0	0
3 (48 hr)	-	*	0	0	0	0	0
12 (3 wk)	-	-	0	0	0	0	0

- No assessment of coordinated was conducted on this day because of possible acute clinical effects from atropine.
* animals sacrificed for NTE and AChe determinations.
[a] Data taken from study report, page 20.

C. Neuropathy Target Esterase (NTE) Studies: No biologically significant effects were seen in any of the 3 birds/group tested in the study (Table 5).

Table 5: Results from the study of NTE in nerve tissue at 24 and 48 hours after treatment from 3 hens/group [a]

(Hr after treatment)/Nerve tissue	Right brain		Spinal cord		Sciatic nerve	
	NTE activity	Percentage inhibition	NTE activity	Percentage inhibition	NTE activity	Percentage inhibition
(24 hr) Control	2651	-	689	-	152	-
(24 hr) 40 mg/kg	2601	2%	684	0.7%	155	-2%
(48 hr) Control	2686	-	734	-	141	-
(48 hr) 40 mg/kg	2579	4%	678	8%	150	-6%

[a] Data taken from study report, page 39.

C. Cholinesterase Activity: Cholinesterase activity (% inhibition) was studied in the left half of the brain from 3 hens/group killed 24 and 48 hours after treatment. Brain cholinesterase was inhibited 83% at 24 hours and 59% at 48 hours after treatment (Table 6).

Table 6: Cholinesterase activity in left brain tissue from the NTE study groups [a]

Group	Hours post treatment	Cholinesterase activity (% inhibition)
Vehicle control	24 48	24.55 (-%) 24.55 (-%)
40 mg/kg	24 48	4.09 (83%) 10.12 (59%)

[a] Data taken from study report, page 21.

B. Body weights and body weight gains: - Mean body weight, which had decreased 90 g by day 7, slowly recovered to a decrease of 60g by day 14 and by 20 g by day 21. The decrease in body weight a day 7 appeared to be treatment related (Table 7).

Table 7: Body weights in kg and weight gain in g at intervals after treatment.[a]

Group/Days after treatment	Body weight (kg)			
	Day 0	Day 7	Day 14	Day 21
Males				
0	1.63	1.69	1.68	1.70
40 mg/kg	1.69	1.60	1.63	1.67
	Body weight gain (g)			
40 mg/kg	0	-90	-60	-20

a Data obtained from the study report, page 19. Body weight gain calculated by the reviewer.

2. Gross pathology - No findings were seen at gross necropsy.

3. Microscopic pathology: At necropsy at 3 weeks after treatment, no evidence of neuropathy was seen in treated birds. A single incidence of focal gliosis was seen in each of the cerebrum, cerebellum and brain stem with one incidence in the cerebrum of controls. Round cell infiltration grade 1 were seen in the cerebrum from controls and treated groups with no treatment relationship. Round cell infiltration was seen at slightly higher incidence in sciatic nerve distal and proximal treatment groups (50% vs. 22% in controls and 67% vs. 55% in controls, respectively) , but neither were considered to be treatment related (Table 8). The total number of histological lesions seen divided by the number of birds examined in controls and treated groups did not differ. The microscopic examination did not suggest any organophosphate induced delayed neuropathy (OPIDP) in this hen study.

Table 8: Results of microscopic examination of nervous tissue from hens acutely dosed with disulfoton and examined 3 weeks after dosing.[a]

Tissue	Vehicle control	40 mg/kg
Number hens in examined	9	12
Olfactory region Round cell infiltr. - grade 1	0	1 (8%)
Cerebrum -round cell infiltr. - grade 1 -focal gliosis - grade 1	4 (44%) 1 (11%)	3 (25%) 1 (8%)
Cerebellum -focal gliosis - grade 1	0	1 (8%)
Brain stem -focal gliosis - grade 1	0	1 (8%)
Sciatic nerve - dist. -focal round cell Infiltr - grade 1	2 (22%)	6 (50%)
Sciatic nerve - prox.. -degener. nerve fibers - grade 1 -focal round cell Infiltr - grade 1	3 (33%) 5 (55%)	2 (17%) 8 (67%)
Spinal cord, cervic. -round cell Infiltr. - grade 1	1 (11%)	0
Spinal cord, thorac. -round cell infiltr. - grade 1	2 (22%)	3 (25%)
Spinal cord, lumbar -round cell Infiltr. - grade 1	2 (22%)	0
Total incidence of histological findings/# bird studied (all grade 1, minimal)	22/9 = 2.4	26/12 = 2.2

[a] = Data obtained from the submitted report, page 57 and 58.

III. DISCUSSION

A. Investigator's conclusions - Body weights were considered to be decreased at day 7 after dosing, but the birds slowly recovered. By week 3 body weight was not different from control body weight. The study showed no OPIDP from a dose level of 40 mg/kg of disulfoton in this 3 week study. A previous study showed an LD50 of 28 mg/kg in hens. The results were consistent with the previously submitted acute delayed neurotoxicity study in hens. The uncoordinated gait seen for the first 2 days of the study was caused by the atropine administered and can not be attributed to administration of disulfoton.

B. Reviewer's discussion - This reviewer agrees with the conclusions of the report author that no evidence of OPIDP was seen in this adequately conducted study. The study was conducted at a sufficiently high dose level to detect any delayed neuropathy in hens. The 3 (25% - 8% in each region) lesion found in treated birds and 1 (11%) in control brain regions were similar to those found in controls from previous studies and were considered incidental.

C. Study deficiencies - No deficiencies were found.